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Ariosa Diagnostics, Inc.

UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

SAN FRANCISCO DIVISION

ARIOSA DIAGNOSTICS, INC.,

Plaintiff,

vs.

SEQUENOM, INC.,

Defendant.

SEQUENOM, INC.,

Counterclaim Plaintiff,

vs.

ARIOSA DIAGNOSTICS, INC.,

Counterclaim Defendant,

and

ISIS INNOVATION LIMITED,

Nominal Counterclaim Defendant.

Case No. 3:11-cv-06391-SI

DECLARATION OF ERIC R. FEARON IN SUPPORT OF ARIOSA DIAGNOSTICS, INC.'S OPPOSITION TO SEQUENOM, INC.'S MOTION FOR PRELIMINARY INJUNCTION

Date of Hearing: June 15, 2012
Time of Hearing: 9:00 a.m.
Location: Courtroom 10
19th Floor

Judge: Hon. Susan Illston

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I, Dr. Eric R. Fearon, declare as follows:

I. Introduction

1. I am the Emanuel N. Maisel Professor of Oncology and Professor of Internal Medicine, Human Genetics, and Pathology at the University of Michigan Medical School. I also serve as Chief of the Division of Molecular Medicine & Genetics in the Department of Internal Medicine, and Associate Director for Basic Science, Deputy Director, and co-leader of the Cancer Genetics Program in the University of Michigan Comprehensive Cancer Center. I am both an M.D. and a Ph.D. I have thirty years of experience in the fields of genetics and cancer diagnosis.

2. I have been retained by Ariosa Diagnostics, Inc. ("Ariosa") to provide my expert opinion in connection with litigation between Ariosa and Sequenom, Inc. ("Sequenom") regarding U.S. Patent No. 6,258,540 (the "'540 patent"). I have been asked to evaluate and provide opinions on the science and claims in the patent, including the validity of claims 1, 2, 8, 19-22, 24, and 25 of the '540 patent. My declaration is submitted in support of Ariosa's opposition to Sequenom's Motion for Preliminary Injunction.

3. I have not previously testified as an expert at trial or by deposition in any case. I am being compensated at an hourly consulting rate of $400 for my work on this matter. My compensation is not dependent on the outcome of this case.

II. Professional Experience and Qualifications

4. I have pursued and/or directed laboratory research using molecular and cell biological and genetics approaches to investigate various issues in cancer development and human disease pathogenesis for thirty years. A copy of my Curriculum Vitae, which includes a list of my published research articles, review/editorial articles, and book chapters is attached as Exhibit 1.

5. I received a B.A. degree in Biophysics in 1983, and M.D. and Ph.D. degrees in Biology/Human Genetics in 1990, all from Johns Hopkins University.

6. Following two years of post-doctoral fellowship in the Division of Hematology, Department of Medicine and the Oncology Center at Johns Hopkins University School of Medicine, I was appointed as an Assistant Professor of Pathology and Biology at Yale University in 1992.

7. I joined the faculty at the University of Michigan in 1995, where I was appointed as the Emanuel N. Maisel Professor of Oncology and Associate Professor of Internal Medicine in the Division of Molecular Medicine and Genetics, with joint (secondary) appointments in the Departments of Human Genetics and Pathology. I was also appointed as the Associate Director for Basic Science in the University of Michigan Comprehensive Cancer Center in 1995. In 2001, I was promoted to the rank of Professor in the Departments of Internal Medicine, Human Genetics, and Pathology.

8. During my seventeen-year tenure at the University of Michigan Medical School, I have taken on additional leadership roles, including as Deputy Director of the Cancer Center in 2005 and Chief of the Division of Molecular Medicine & Genetics in the Department of Internal Medicine in 2010.

9. My research career using molecular biology approaches to address human disease pathogenesis was initiated in pediatric genetics laboratories at Johns Hopkins Medical School, where I participated in investigating DNA sequence variations and gene mapping in the human population (Prochownik et al. N Engl J Med 1983; Antonarakis et al. Proc Natl Acad Sci USA 1983; Fearon et al. Am J Hum Genet 1984), as well as investigations of the molecular basis of a novel form of β-thalassemia (Fearon et al. Blood 1983) and urea cycle disorders (Fearon et al. Hum Genetics 1985). Throughout my career, my laboratory colleagues and I have used a wide range of nucleic acid analysis technologies in our research efforts.

10. I have authored more than 115 full-length research articles in peer-reviewed journals, 33 review or editorial articles, and 24 book chapters. The Thompson Reuters ISI Web of Science indicates that the papers that I have authored have received more than 32,000 citations, offering robust evidence of the scientific impact of my published work.

11. I currently serve on the editorial board or as an editor of nine scientific journals, namely: The Journal of Clinical Investigation; Current Biology; Cancer Research; Genes, Chromosomes & Cancer; Neoplasia; Molecular Cancer Research; Clinical & Translational Science; Translational Oncology; and Gastroenterology.

12. Besides serving on the editorial boards of the above listed journals, from the early and mid-1990's in some cases, I previously served on the editorial boards at Human Molecular Genetics (1996-2003), Laboratory Investigation (1996-2005), Clinical Cancer Research (1996-2004), and Journal of Biological Chemistry (2005-2010).

13. As a result of my extensive service as an editor or editorial board member and as a journal reviewer for various high impact general interest journals (*e.g.*, Science, Nature, Cell), genetics journals (*e.g.*, Nature Genetics), and medical journals (*e.g.*, Nature Medicine, New England Journal of Medicine), I have acquired in-depth knowledge of the revolution in molecular analysis of nucleic acids and how it has impacted diagnostic approaches in various fields in medicine over the past twenty years.

14. Over the past eighteen years, I have served as a principal investigator or co-investigator on grants from the National Institutes of Health ("NIH") and other funding agencies. In addition, I have an extensive record of service on various scientific review committees at the NIH and other funding agencies, including serving as the Chair of the NIH Pathology B Study Section (2001-2003) and the NIH Cancer Genetics Study Section (2003-2004). I also have served as a member of a number of federal scientific advisory and oversight committees, including the following: the Panel to Assess the NIH Investment in Research on Gene Therapy (1995), the National Cancer Institute ("NCI") Developmental Diagnostics Working Group (1996-1997), the NCI Board of Scientific Advisors, and the National Genome Research Institute Scientific Planning Subcommittee (1997-1998). Besides these federal advisory roles in the cancer, gene therapy, and genome research areas, I have served and continue to serve on cancer center and other medical advisory boards at academic medical institutions around the U.S.

15. I served on the scientific advisory boards of Onyx Pharmaceuticals from 1992 to 2002 and Exact Laboratories from 1996 to 1999. Onyx Pharmaceuticals is a molecular oncology therapeutics company, best known for its development of the FDA-approved anti-cancer agent Sorafenib. Exact Sciences (formerly Exact Laboratories) is recognized for its pioneering work in the use of cell-free DNA in stool for colon cancer screening.

16. I have received various honors for my research accomplishments, including election to the American Society of Clinical Investigation, the Association of American Physicians, and the Johns Hopkins Society of Fellows; the presentation of named lectureships at academic institutions in the U.S. and Canada; election to the Board of Directors for the American Association for Cancer Research; and co-organizer or Chair for major international research conferences in the cancer biology and cancer genetics fields. I was also elected to serve as Vice-President, President-Elect, and President of the American Society for Clinical Investigation during the period 2003-2006.

III. Basic Principles of Genetics

A. Structure of Nucleic Acids

17. Nucleic acids are long molecules that are composed of smaller subparts called nucleotides or nucleosides. The nucleotides or nucleosides are held together in a chain composed of alternating sugar and phosphate molecules, as shown in Figure 1. There are two types of nucleic acids, deoxyribonucleic acid ("DNA") and ribonucleic acid ("RNA"). There are four types of nucleotides that make up DNA: adenine, guanine, cytosine, and thymine. They are abbreviated A, G, C, and T. RNA is made up of four nucleosides: adenine, guanine, cytosine, and uracil. They are abbreviated A, G, C, and U.

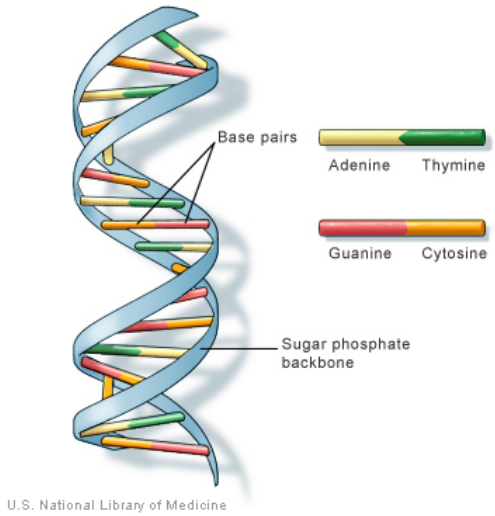

Figure 1

18. DNA is a double stranded molecule in the shape of a helix, and the bulk of the intra-cellular DNA is generally located inside the cell's nucleus. The nucleotides on one strand of the double stranded DNA bond to the nucleotides on the opposing strand. Each nucleotide normally bonds to just one other type of nucleotide. Adenine bonds to thymine, and cytosine bonds guanine. This interaction is referred to as complementary base pairing. Accordingly, one strand of DNA "complements" the opposing strand. For certain DNA regions, the order of nucleotides in DNA, usually referred to as its "sequence," can code for proteins, which are molecules necessary for the human body to develop and function properly. Only a small portion of the total DNA in the human genome codes for proteins. The function of much of the non-coding DNA remains a mystery. Regions of DNA that encode proteins (or that produce certain functional RNAs in the cell) are typically referred to as genes.

19. RNA is a single stranded molecule. A major fraction of the RNAs in cells, termed messenger RNAs ("mRNAs"), convert information encoded in DNA into proteins. In simplest terms, the process whereby a cell makes a protein from DNA involves two steps, commonly referred to as "transcription" and "translation." When a gene coding for a particular protein is active, the two strands of DNA locally unwind. In the "transcription" step, chemical mechanisms inside the cell read the code in one of those DNA strands and produce an mRNA that contains the code for directing the production of the same protein. Then, in the "translation" step, other cellular mechanisms read the code in the mRNA strand and produce the relevant protein.

B. Chromosomes

20. DNA is tightly coiled inside the nucleus of the cells, forming dense structures known as chromosomes. This is illustrated in Figure 2. The DNA in human cells is normally arranged on 23 pairs of chromosomes, for a total of 46 chromosomes. A person typically inherits one copy of each chromosome from the mother, and one copy of each chromosome from the father.

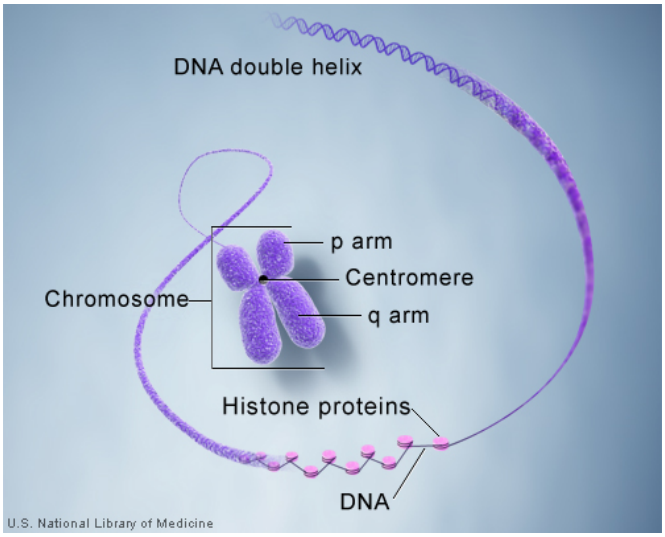

Figure 2

21. One pair of chromosomes determines a person's sex. The sex chromosomes are referred to as the X chromosome and the Y chromosome. Females have two X chromosomes, whereas males have an X and a Y chromosome.

22. The other 22 pairs of chromosomes, which are numbered 1 through 22, are referred to as "autosomes." The autosomes contain the remainder of the genetic information.

23. Each person typically inherits one copy of chromosomes 1-22 and an X chromosome from the mother, and one copy of chromosomes 1-22 and an X or a Y chromosome from the father. Human cells with intact 23 chromosome pairs and no major sub-chromosomal defects are said to be "euploid," meaning that they have the normal number of chromosomes for the species.

24. Egg cells normally contain one copy of each of the autosomes and a single X chromosome, while sperm cells normally contain one copy of each of the autosomes and either an X or a Y chromosome. Thus, when a sperm fertilizes an egg, the resulting cell, called a "zygote," will have a total of 46 chromosomes, half from the mother and half from the father.

25. Each gene occurs at a particular location ("loci" or "locus" when referring to a single location) on each chromosome pair. However, the genes inherited from the mother and the father at a particular locus are not always identical; the nucleic acid sequence at any given locus might be different. These different forms of a particular gene are called alleles. A person has two

alleles for every gene—one allele resides on the chromosome inherited from the mother, and the other allele resides on the chromosome inherited from the father. Differences in the nucleic acid sequences of different alleles are termed variations or variant alleles. If the DNA sequence variations are common in a population, the variations are often called DNA "polymorphisms." If the DNA sequence variations play a role in disease or predisposition to a disease, the sequence variations are often called "mutations."

C. Down Syndrome

26. Alterations can occur in which a person will have more than or less than two copies of an autosome or the sex chromosomes. These are known as "aneuploidies." When there is an extra copy of a chromosome, such that there are three copies of the chromosome, this is called "trisomy."

27. Down syndrome is usually caused by having three copies of chromosome 21, and is sometimes called "trisomy 21." In more than 90% of cases the extra chromosome is inherited from the mother (Antonarakis et al. N Engl J Med 1991). In the remaining cases, the extra chromosome is inherited from the father. In a very small fraction of cases, Down syndrome is caused by a "translocation" of chromosome 21, meaning that a portion of chromosome 21 becomes attached to another chromosome. When the fetus inherits two normal copies of chromosome 21, plus the chromosome with the translocation, this can cause Down syndrome. Translocations of chromosome 21 are more often inherited from the mother than from the father.

28. Sperm and egg cells are created in the human body through a process called meiosis. During meiosis, the two copies of each chromosome in the cell are separated into different cells. Sometimes, the two copies of each chromosome do not separate correctly, resulting in egg or sperm cells that contain two or zero copies of a particular chromosome instead of one. This is called nondisjunction. When an egg or sperm cell that contains two copies of chromosome 21 is fertilized by an egg or sperm cell with one copy of chromosome 21 (the normal number for an egg or sperm cell), the fetus will have three copies of chromosome 21. Nondisjunction events occur much more frequently in egg cells.

D. Cell-Free Nucleic Acids

29. Blood is made of blood cells suspended in liquid called plasma. Plasma is made up of water, proteins, glucose, clotting factors, mineral ions, hormones, and carbon dioxide. Serum is plasma without clotting factors.

30. Most nucleic acids in blood are located within the circulating cells in the bloodstream. However, at least since the 1940's, it has been known that some DNA, which is a nucleic acid, is not located inside the cells, but rather circulates free of cells in the plasma and serum (Mandel & Metais Proc Meetings Biol Soc 1948; Tan et al. J Clin Invest 1966; Kamm & Smith Clin Chem 1972; Leon et al. Cancer Res 1977). Such extracellular nucleic acid in the plasma and serum is referred to as "cell-free" nucleic acid.

31. The discovery of the natural phenomenon of cell-free nucleic acid opened up the possibility of using plasma and serum to test for various genetically based diseases and conditions.

32. The plasma and serum of pregnant women contains cell-free fetal nucleic acid. This natural phenomenon has led to the use of maternal plasma and serum to test for genetically based diseases and conditions in the fetus. Because these tests only require a maternal blood sample, and are therefore not invasive to the fetus, they are often referred to as "noninvasive" tests.

IV. The '540 Patent

33. The '540 patent is titled "Non-Invasive Prenatal Diagnosis," and lists as inventors Yuk-Ming Dennis Lo and James Stephen Wainscoat. The '540 patent is attached as Exhibit 2 to this declaration.

34. The patent lists March 4, 1997 under "Foreign Application Priority Data."

35. The patent generally claims methods for "amplifying" and "detecting a paternally inherited nucleic acid of fetal origin" in "a maternal serum or plasma sample." (Ex. 2, Claim 1 at 23:61-67.)

36. I understand that Sequenom alleges that Ariosa infringes at least claims 1, 2, 8, 19-22, 24, and 25 of the '540 patent.

V. Materials Reviewed

37. I have reviewed the '540 patent and its prosecution history, the prosecution history of U.S. Patent Application No. 09/872,063, Sequenom's Motion for Preliminary Injunction and supporting papers, the declaration of Dr. Farideh Bischoff in support of Ariosa's opposition to Sequenom's Motion for Preliminary Injunction, and additional published papers in the biomedical literature that are cited herein.

38. The opinions that I offer in this declaration are based on my knowledge obtained from my professional experiences in research as well as from critical review of the documents and published findings cited in this declaration. If I am provided with additional materials relevant to the case, such as additional testimony or other discovery material, I may revise or modify my opinions to reflect the impact of the new materials on my scientific opinions. As such, I reserve the right to update the scientific opinions and conclusions that I offer based on such new information.

VI. Person of Ordinary Skill in the Art

39. I understand that a person of ordinary skill in the art is a person of ordinary skill, not one of extraordinary skill, in the field of the invention at the time of the invention. I further understand that, in the absence of other information, the time when an invention was made is generally considered to be the date on which the patent application for the invention was filed. In this case, I understand that the '540 patent lists March 4, 1997 under "Foreign Application Priority Data." Thus, for purposes of my analysis, I have used March 4, 1997 as the time the invention embodied in the '540 patent was made.

40. I have been asked to provide my opinion as to what level of training and experience a person of ordinary skill in the art would have had in 1997.

41. The '540 patent concerns detection of paternally inherited nucleic acids, which involves use of techniques and procedures that are generally within the fields of molecular biology, molecular genetics, and biochemistry.

42. It is my opinion that a person of ordinary skill in the art for the technology of the '540 patent would have a doctoral degree in a field of molecular biology with knowledge of

molecular genetics and biochemistry. The person of ordinary skill in the art would have experience with the handling, isolation, and analysis of nucleic acids, including nucleic acids from blood samples.

VII. Summary of Opinions

43. I am not offering an opinion on claim construction, but I have reviewed Sequenom's constructions. I have also read the declaration of Dr. Bischoff in support of Ariosa's opposition to Sequenom's Motion for Preliminary Injunction, which is attached as Exhibit 3. Based on my own reading of the patent and the prosecution history, including the prosecution history of U.S. Patent Application No. 09/872,063, I agree with the claim constructions Dr. Bischoff proposes.

44. It is my opinion that, under both Ariosa's and Sequenom's claim constructions, claims 1, 2, 8, 19-22, 24, and 25 of the '540 patent describe no more than a law of nature coupled to well-understood, routine, and conventional steps already engaged in by the scientific community as of 1997, that, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately. It is therefore my opinion that the claims are invalid.

45. It is my opinion that the '540 patent specification does not contain sufficient disclosure to enable a person of skill in the art in 1997 to use the methods in claims 1, 2, 8, 19-22, 24, and 25 to detect aneuploidies in the fetus without undue experimentation. It is therefore my opinion that the claims are invalid.

46. It is my opinion that the paper by Kazakov and his colleagues (Kazakov et al. Tsitologiia 1995), attached as Exhibit 4, inherently anticipates claims 1, 2, 8, 19-22, 24, and 25 under Sequenom's claim constructions, and any other claim constructions broad enough to encompass any method which amplifies and detects a paternally inherited nucleic acid, irrespective of whether the method is able to discriminate, enrich, identify, or isolate which nucleic acids are paternally inherited. It is therefore my opinion that the claims are invalid.

47. I reserve the right to analyze the claims under any different claim construction at another stage of the case.

VIII. General Legal Standards

48. For purposes of my analysis, I have been asked to assume by counsel that the following legal standards apply.

49. I understand that the following requirements of patentability or patentability factors—patentable subject matter, enablement, and novelty/anticipation—may be considered in determining whether the claims of a patent are valid or not. I also understand that the claims of an issued patent are presumed valid, but those claims can be shown to be invalid by clear and convincing evidence that they fail to comply with one or more requirements of patentability. I further understand that when considering a motion for preliminary injunction, clear and convincing evidence is not the standard that is applied to the invalidity analysis. Instead, if there is a substantial question concerning the validity of the patent—meaning an invalidity defense that the party asserting the patent has not shown to lack substantial merit—then the party asserting the patent has not shown a likelihood that it will succeed on the merits.

50. I understand that the scope of what is covered by a patent claim depends on how the language of the claim is interpreted. I further understand that parties in litigation will often propose definitions, called "claim constructions," for the terms in a patent claim. I also understand that opposing parties will often disagree on these claim constructions.

51. I understand that Sequenom has set forth its proposed claim constructions in its Memorandum of Points and Authorities in support of its Motion for Preliminary Injunction and in the Declaration of Dr. Mark I. Evans in support of that motion. I understand that Sequenom proposes the following claim constructions: (1) a "'nucleic acid' is simply a sugar and phosphate backbone with a string of bases (A, T, G, and Cs)"; (2) a "'paternally inherited nucleic acid of fetal origin' is a nucleic acid that originates from the fetus and is inherited from the father"; (3) to "'detect' a nucleic acid means the same thing it means in everyday ordinary language, that is, to discover or determine the existence, presence, or fact of it"; (4) "'[a]mplifying' a nucleic acid likewise has its everyday meaning, that is, to increase the amount of the nucleic acid by making copies of it"; and (5) a "'maternal serum or plasma sample' is a sample of a portion of blood taken

from the mother; the liquid, non-cellular portion made of water, salts, and protein is called 'plasma,' and when plasma's clotting factors are removed, it is called 'serum.'"

IX. Whether the '540 Patent Describes More than a Law of Nature

52. For purposes of this analysis I have applied Sequenom's proposed claim constructions and Ariosa's proposed claim constructions. Consideration of either set of claim constructions leads to the same conclusion—the asserted claims simply couple a natural phenomenon or law of nature to well-understood, routine, and conventional nucleic acid processing and analysis technologies previously used by researchers in the field. Indeed, I do not believe any construction of the claims could lead to a different conclusion, as demonstrated by my analysis below. Therefore, it is my opinion that the claims are invalid.

A. Patentable Subject Matter Requirement

53. For purposes of my analysis, I have been asked to assume by counsel that the following legal standards apply.

54. I understand that laws of nature, natural phenomena, and abstract ideas are not patentable subject matter.

55. I understand that the application of a law of nature, natural phenomena, or abstract idea may be patentable.

56. I understand that if the steps in a claimed method (apart from the laws of nature, natural phenomena, or abstract ideas themselves) consist of well-understood, routine, conventional activities already engaged in by the scientific community that, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately, then the method claim is not patentable.

57. I understand that an additional reason to find a patent violates the patentable subject matter requirement is that a patent inhibits further innovation by tying up too much future use of a law of nature, natural phenomenon, or abstract idea.

B. The '540 Patent Describes a Law of Nature Coupled to Conventional Steps

58. The '540 patent specification explains that "[i]t has now been discovered that foetal DNA is detectable in maternal serum or plasma samples." (Ex. 2 at 1:50-51.) It characterizes this

as "a surprising and unexpected finding . . . ." (Ex. 2 at 1:51-52.) Regardless of how "unexpected" or "surprising" this finding may have been, which in my opinion it was not, the presence of cell-free fetal nucleic acid in maternal serum or plasma is a natural phenomenon or law of nature. Because half of all nucleic acid is inherited from the father and half is inherited from the mother, fetal nucleic acid will have by definition a paternal contribution. Thus, it is also a natural phenomenon or law of nature that paternally inherited nucleic acid of fetal origin will be present in maternal serum or plasma samples. Moreover, because paternally inherited nucleic acid of fetal origin is present in maternal serum or plasma of a pregnant female, it follows that such nucleic acid could be amplified and then detected in maternal serum or plasma samples from a pregnant female using conventional techniques known to those skilled in the art.

59. The '540 patent describes various techniques to analyze cell-free fetal nucleic acid in maternal plasma and serum. However, each of those techniques was conventional as of 1997.

60. My observation that all of the techniques described in the '540 patent were conventional is consistent with the specification's characterization of the findings that follow from the examples: "Maternal plasma, therefore, offers an easily accessible foetal DNA source for prenatal genetic analysis." (Ex. 2 at 16:40-42.) The cell-free fetal DNA is "easily accessible" because it can be detected and analyzed according to techniques which were well-understood , and in many cases commercially available, as of 1997.

61. Furthermore, statements made in the prosecution history of the '540 patent, which I understand are relevant to interpreting the scope of what is claimed, are consistent with the notion that the only "invention" was the discovery of cell-free fetal nucleic acid in maternal plasma and serum, and everything else already existed in the scientific literature. For example, the applicants argued that:

> One skilled in the art could take advantage of the present application describing the presence of foetal DNA in the plasma or serum and apply it to the detection of paternally-inherited non-Y sequences in addition to those which are described.

(Reply to Examiner's Action dated April 18, 2000, Ex. 5 at pp. 5-6.)

62. The applicants also stated that:

> [T]he present invention results in the new identification that foetal DNA is present in maternal plasma or serum. Many of the points highlighted by the Examiner would be considered to be a matter of routine experimentation to one skilled in the art of DNA detection, to identify the most appropriate technique for a particular required diagnosis. The person skilled in the art has a broad range of techniques available for the detection of DNA in a sample.

(Reply to Examiner's Action dated April 18, 2000, Ex. 5 at p. 10.)

63. The applicants urged that the '540 patent specification should be considered sufficient to enable scientists to practice the recited methods, because the DNA analysis methods were "routine" and "well known" techniques:

> Although there are a wide variety of different types of polymorphisms which could be detected in connection with the present application, such polymorphisms and techniques for analysis of DNA **are simply a matter of routine** for one skilled in the art. Therefore, it is not necessary for the Applicants to set out each of the many ways in which DNA might be analyzed. The description is sufficient simply by instructing one skilled in the art to carry out a suitable analysis. . . . [O]ne skilled in the art is readily able to apply the teachings of the present application to any one of the **well known techniques** for detection of DNA with a view to analysis of foetal DNA in paternal [sic] plasma or serum.

(Reply to Examiner's Action dated April 18, 2000, Ex. 5 at pp. 7-8 (emphasis added).)

64. In her statement of reasons for allowance of the '540 patent, the examiner showed that she too understood the "invention" to be the discovery of cell-free fetal nucleic acid in maternal plasma and serum:

> The closest prior art is directed to detecting alterations in plasma DNA for diagnosing and or monitoring the development of DNA (Stroun et al GB 2299166, September 1996). The art also teaches detecting fetal cells in maternal blood and performing diagnostic tests on the blood. However, **the art does not teach nor reasonably suggest that nucleic acid of fetal origin is present in maternal serum or plasma**.

(Notice of Allowability, Ex. 6 at p. 3 (emphasis added).)

65. The prosecution history of U.S. Patent Application No. 09/872,063, which I understand is a continuation of the '540 patent, and therefore also relevant to interpreting the scope of the invention, further shows that the "invention" was the discovery of cell-free fetal nucleic acid in maternal plasma and serum. For example, the applicants argued:

> The instant application clearly teaches that large amounts of fetal nucleic acid are present in maternal serum or plasma from the first

> trimester and can be detected. The identification of such large amounts of fetal nucleic acid is, **in itself**, the solution to a significant technical problem, namely, how to obtain, non-invasively, analytically useful amounts of fetal nucleic acid for genetic analysis.

(Reply to Official Action dated November 16, 2001, Ex. 7 at p. 6 (emphasis added).)

66. The applicants also stated that "**the critical teaching of the present invention** is that ALL fetal DNA is greatly increased in concentration in the maternal plasma or serum as compared with maternal whole blood" (Reply to Official Action dated November 16, 2001, Ex. 7 at p. 10 (emphasis added)) and that "[t]he use of fetal nucleic acid analysis for a wide variety of diagnostic and other purposes is **well known in the art**" (*id.* at p. 6 (emphasis added)).

67. In support of the continuation application, Dr. Lo himself submitted an affidavit that explained in some detail that he considered his "invention" to reside in the discovery of cell-free fetal nucleic acid in maternal plasma and serum. For example, he stated:

> In summary, **the fundamental teachings of the present patent application** are (1) that the detection of fetal DNA in maternal plasma or serum is easier and more reliable than in whole peripheral blood (or the cellular fraction thereof), and (2) that sufficient DNA is present in the maternal serum and plasma, even during the first trimester of pregnancy, to enable the detection of fetal DNA from any chromosomal locus.

(Declaration of Dr. Lo under Rule 132, Ex. 8 at ¶ 5 (emphasis added).)

68. Dr. Lo further stated:

> I believe . . . that when the competent person in the field has been taught, **by the present invention**, that the maternal plasma or serum contains sufficient fetal DNA in the high concentrations found, he would have no difficulty in carrying out fetal diagnosis to detect even small genetic defects, in any chromosomal locus, and even in the first trimester of pregnancy. **Suitable amplification techniques can be ordinary PCR or more sophisticated developments thereof, but these techniques were all known in the literature before the date of my invention.**

(Declaration of Dr. Lo under Rule 132, Ex. 8 at ¶ 7 (emphasis added).)

69. This is consistent with statements made by Dr. Evans, who submitted a declaration in support of Sequenom's Motion for Preliminary Injunction. During his deposition, Dr. Evans admitted that cell-free fetal DNA was not thought to be in maternal plasma, but we now understand that it was always there. (Evans Deposition Transcript, Ex. 9 at 167:14-25.) He also

admitted that the polymerase chain reaction, as well as "other methodologies which could be used" for amplifying were known in 1997 (*id.* at 150:18-151:7), as were methods for isolating plasma (*id.* at 152:4-7). When asked "[o]nce you know where you want to look for fetal DNA in the material—in the maternal plasma, as an example from—per Dr. Lo's 1997 paper, the techniques for how to look for that DNA, how would one of skill in the art know of those techniques at that time?" (*Id.* at 155:23-156:3.) Dr. Evans replied "[t]echniques such as the polymerase chain reaction were known in the field at that point," which could be used to detect DNA. (*Id.* at 156:5-11.)

70. Dr. Evans unambiguously admitted that creating plasma and serum samples, and amplifying and detecting nucleic acids were routinely performed together prior to 1997:

> Q. Others before Dr. Lo amplified and detected nucleic acids, right?
>
> A. Yes.
>
> Q. In fact, traditional DNA diagnostics well before 1997 traditionally involved three steps, right: Sample preparation, amplification, and detection, correct?
>
> A. Commonly.
>
> Q. And others before Dr. Lo amplified and detected nucleic acid in plasma and serum, true?
>
> A. Yes

(Evans Deposition Transcript, Ex. 9 at 188:5-17.)

71. I have been instructed that the claims define the subject matter covered by a patent and that it is accordingly necessary to consider whether each claim asserted against Ariosa recites more than a law of nature or a natural phenomenon coupled with methodologies that were conventional. I have been asked to perform this analysis as to claims 1, 2, 8, 19-22, 24, and 25. My analysis is below. I have also prepared a chart containing my analysis as to each of these claims, which is attached to this declaration as Exhibit 16.

1. Claim 1

Claim 1 reads as follows:

> 1. A method for detecting a paternally inherited nucleic acid of fetal origin performed on a maternal serum or plasma sample from a pregnant female, which method comprises
>
> amplifying a paternally inherited nucleic acid from the serum or plasma sample and
>
> detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample.

72. This claim involves "amplifying" and "detecting" a paternally inherited nucleic acid of fetal origin in "a maternal serum or plasma sample."

73. As of 1997, separating serum or plasma from blood to create a sample for nucleic acid analysis was a well-understood, routine, conventional activity previously engaged in by the scientific community (Leon et al. Cancer Res 1977; Shapiro et al. Cancer 1983; Vasioukhin et al. Br J Haematol 1994; Sorenson et al. Cancer Epi Biomarkers 1994; Chen et al. Nat Med 1996; Nawroz et al. Nat Med 1996). Indeed, the '540 patent specification refers to "standard techniques" for "preparation of serum or plasma from the maternal blood sample." (Ex. 2 at 2:26-27.) These standard techniques include using a centrifuge to separate the cells in the blood from the liquid component, followed by extracting (*i.e.*, purifying the DNA by removing proteins, often referred to as "isolating" the DNA) the DNA, sometimes using a commercial DNA extraction kit (*id.* at 4:38-51); controlled heating (*id.* at 2:31-32); and using an enzyme called "proteinase K" followed by either the chemicals phenol and/or chloroform to purify DNA from the sample (*id.* at 2:32-34). All of these techniques were well-understood, routine, and conventional as of 1997. There is no plasma or serum or DNA preparation technique disclosed in the '540 patent specification that was not well-understood, routine, and conventional as of 1997.

74. Claim 1 broadly requires the step of "amplifying a paternally inherited nucleic acid," and that was well-understood, routine, and conventional as of 1997. The specification acknowledges that "[s]tandard nucleic acid amplification systems can be used" to amplify fetal nucleic acid in the serum or plasma sample, "including PCR, the ligase chain reaction, nucleic acid sequence based amplification (NASBA), branched DNA methods, and so on." (Ex. 2 at 2:43-48.) PCR is an acronym for the polymerase chain reaction. In this amplification method, DNA, which is double stranded, is separated into two strands using heat, which disrupts the bonds between the

nucleotides on each strand. Next, enzymes are used along with short DNA fragments that are necessary to begin the amplification process (called "primers"), and free nucleotides to build two new strands of DNA that complement the old strands (often referred to as "template" strands). The process is then repeated, starting with separation of the strands of all DNA molecules in the mixture—both old and new—through a heating step, followed by the priming and amplification step, resulting in essentially geometric amplification of the DNA target sequences. The ligase chain reaction is similar to PCR in that it uses cycles of separating the DNA through heating and amplifying the DNA using enzymes. However, two primers are used that bond to adjacent locations on a strand of DNA, and then an enzyme known as a ligase bonds the two primers together. Nucleic acid sequence based amplification (NASBA) is a technique for amplifying RNA using primers, nucleotides, and enzymes at a constant temperature. Branched DNA methods involve the use of a number of short DNA fragments that interact with the target nucleic acid. But branched DNA methods do not increase the number of copies of the target nucleic acid. Instead, they are used for detection of a target nucleic acid. Regardless of the approach listed, all of these methods were well-understood, routine, and conventional as of 1997. There is no amplification method disclosed in the '540 patent specification that was not well-understood, routine, and conventional as of 1997.

75. Claim 1 broadly describes the next step as "detecting the presence of a paternally inherited nucleic acid," and many techniques for doing this were well-understood, routine, and conventional as of 1997. These included gel electrophoresis, which often involves passing an electric current through a rectangular piece of porous "gel" material. The gel is usually made of a polymerized matrix of agarose or polyacrylamide. The nucleic acid sample is placed at the top of the gel, and the electric current causes the nucleic acid molecules to move through the gel in a fashion dependent on their size. Larger molecules move more slowly than smaller molecules. Thus the molecules begin to separate by size. Once there is sufficient separation, the electric current is switched off. The nucleic acid molecules, which are not visible to the human eye, are then subjected to a "staining" procedure, so that they can be seen in the gel. Ethidium bromide is a chemical often used to stain nucleic acid molecules in a gel, because it binds to the nucleic acid

molecules. Moreover, it will cause the nucleic acid molecules to fluoresce upon exposure to ultraviolet light. Real time quantitative PCR ("Q-PCR") was another well-understood, routine, and conventional method of detecting nucleic acid in 1997. In this technique, nucleic acid is amplified using PCR and the amplified nucleic acid is quantified in real time, *i.e.*, as the amplification is taking place, generally through the use of a fluorescent dye that attaches to the newly generated nucleic acid. Gel electrophoresis and Q-PCR are disclosed in the '540 patent as methods for "detecting" paternally inherited nucleic acids. (*See, e.g.*, Ex. 2 at 5:23-25 ("PCR products were analysed by agarose gel electrophoresis and ethidium bromide staining."); 6:37-40 ("Real time quantitative PCR analysis was performed using a PE Applied Biosystems 7700 Sequence Detector (Foster City, Calif., U.S.A.).").) All of the detection methods disclosed in the '540 patent specification were well-understood, routine, and conventional as of 1997.

76. Claim 1 is directed to "paternally inherited nucleic acid." The '540 patent does not disclose any newly identified "paternally inherited nucleic acid." There were many paternally inherited nucleic acid sequences that were known in 1997, such as sequences on the Y chromosome (which could not be maternally inherited because only males possess a Y chromosome). (*See, e.g.*, Ex. 2 at 7:1-3 ("Sequence data for the SRY gene were obtained from the GenBank Sequence Database (accession number L08063).").) Furthermore, it was well-understood at that time that a fetus inherits half of its DNA from its mother and half from its father, and that the father's nucleic acid sequences, *i.e.*, nucleic acid sequences that could be paternally inherited, could be determined through a process known as "genotyping." (*See, e.g., id.* at 3:20-24 ("This application will require the prior genotyping of the father and mother using a panel of polymorphic markers and then an allele for detection will be chosen which is present in the father, but is absent in the mother.").) Also, by 1997, scientists had access to publicly available DNA sequences through the GenBank sequence database.

77. The '540 patent offers five examples to illustrate the "invention."

78. Example 1 begins by describing preparation of maternal plasma and serum samples according to conventional blood preparation techniques. Because the preparation of plasma and serum from blood samples has been such a standard approach in laboratory and clinical medicine

over the past century, research papers describing the preparation of plasma and serum from blood samples have not routinely referenced the standard methods for preparation of plasma or serum for more than half a century. The '540 patent indicates the plasma and serum samples were processed by conventional methods (Emanuel & Pestka Genet Anal Tech Appl 1993), involving brief heating of samples and then centrifugation in a microcentrifuge, which were conventional approaches for processing fluids containing nucleic acids prior to PCR analysis. PCR was then performed according to the techniques described in 1988 by Saiki and colleagues (Saiki et al. Science 1988), using commercial chemicals. DNA sequences were amplified using techniques disclosed in Dr. Lo's own 1990 paper. Detection of PCR products was by standard gel electrophoresis and ethidium bromide staining. All of the other techniques employed in connection with Example 1 were likewise conventional as of 1997.

79. The same can be said of the techniques utilized in Example 2. Serum and plasma samples were collected and prepared with a commercially available kit (QIAamp Blood Kit, Qiagen) and according to the "blood and body fluid protocol" recommended by the manufacturer and described by Chen and colleagues (Chen et al. Nat Med 1996). Q-PCR was performed according to the protocol described by Heid and colleagues (Heid et al. Genome Res 1996) using a commercially available Applied Biosystems 7700 Sequence Detector that was based on the test described by Holland and colleagues (Holland et al. Proc Natl Acad Sci USA 1991) and that was commercially available through Perkin-Elmer. Primers, dyes, and the associated techniques were borrowed from previously published methods (Lee et al. Nucl Acids Res 1993; Livak et al. PCR Methods Appl 1995). The contamination prevention protocols were from previously published articles (Kwok & Higuchi Nature 1989; Longo et al. Gene 1990). The data was analyzed with commercially available Perkin-Elmer software. All of the other techniques employed in connection with Example 2 were likewise conventional as of 1997.

80. Example 3 similarly described conventional and routine acquisition of serum and plasma samples from pregnant women. DNA extraction from the samples was again performed with a commercially available kit (QIAamp Blood Kit, Qiagen) according to the "blood and body fluid protocol" recommended by the manufacturer and described by Chen and colleagues (Chen et

al. Nat Med 1996). Q-PCR was performed using devices and technologies that were commercially available from Perkin-Elmer (Foster City, Calif). Sequences were amplified using primers and techniques that were standard and based on recommendations from the Perkin-Elmer Primer Express software. All of the other techniques employed in connection with Example 3 were likewise conventional as of 1997.

81. Example 4 utilized the same procedures and equipment described above in Example 2, which were well-understood, routine, and conventional as of 1997. The Mann-Whitney U Test, a well-known statistical test, was used in the determination of cell-free fetal DNA concentration. The remaining methods and techniques for analysis employed in connection with Example 4 were also well-understood, routine, and conventional as of 1997.

82. Example 5 relied on the techniques elaborated in Example 2, which were well-understood, routine, and conventional as of 1997, together with a handful of additional protocols that were likewise well-understood and/or commercially available as of 1997. The Mann-Whitney Rank Sum Test and the Wilcoxon Signed Rank Test were used to analyze the data; both were conventional statistical methods at the time.

83. A review of the five examples is consistent with my conclusion that claim 1 recites nothing beyond a law of nature applied through methods which were well-understood, routine, and conventional. In the five examples provided in the ’540 patent, the DNA present in the plasma and serum samples of pregnant females was processed by standard methods and techniques (Emanuel & Pestka Gen Anal Tech Appl 1993; Chen et al. Nat Med 1996), generally aided by use of a commercial “QIAamp Blood Kit” (Qiagen). DNA amplification was then carried out by PCR with standard methods, materials, and equipment credited in the examples to one of the major relevant commercial suppliers at the time, namely Perkin Elmer–Applied Biosystems, along with references to various previously published papers on PCR amplification and detection technologies (Saiki et al. Science 1988; Kwok & Higuchi Nature 1989; Longo et al. Gene 1990; Holland et al. Proc Natl Acad Sci USA 1991; Lee et al. Nucl Acids Res 1993; Livak et al. PCR Methods Appl 1995; Heid et al. Genome Res 1996). Detection of paternally inherited nucleic acids of fetal origin in the maternal plasma or serum was then undertaken with approaches and

methods similar to those used in prior published work on the amplification and detection via PCR-based methods of paternal nucleic acid isolated from the fetal cells present in maternal blood (reviewed in Steele et al. Clin Obstet Gyn 1996). In sum, all of the '540 patent's experimental techniques were well-understood, routine, and conventional at the time the patent application was filed.

84. Amplifying and detecting cell-free nucleic acid in a plasma or serum sample were routinely used together as of 1997. For example, amplifying and detecting cell-free nucleic acid in plasma or serum samples was a technique that was already being used for tumor DNA by 1994 (Vasioukhin et al. Br J Haematol 1994; Sorenson et al. Cancer Epi Biomarkers 1994) and subsequently in 1996 (Chen et al. Nat Med 1996; Nawroz et al. Nat Med 1996), as acknowledged in the '540 patent specification (Ex. 2 at 1:40-43 ("[I]t has been demonstrated that tumour DNA can be detected by the polymerase chain reaction (PCR) in the plasma or serum of some patients (Chen et al 1996; Nawroz et al 1996).")). Thus, it is my opinion that the steps of claim 1, when viewed as a whole, add nothing significant beyond the sum of their parts taken together.

85. Based on my analysis above, it is my opinion that claim 1 of the '540 patent is nothing more than a law of nature coupled to well-understood, routine, and conventional activities already engaged in by the scientific community as of 1997, that, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately. It is therefore my opinion that claim 1 is invalid.

2. Claim 2

Claim 2 reads as follows:

> 2. The method according to claim 1, wherein the foetal nucleic acid is amplified by the polymerase chain reaction.

86. This claim limits the amplification step to polymerase chain reactions, PCR. Otherwise it is the same as claim 1, and the same analysis applies.

87. As discussed above, PCR was a well-understood, routine, and conventional process as of 1997. Even the particular PCR techniques discussed in the '540 patent were well-understood, routine, and conventional as of 1997.

88. For the reasons stated under claim 1, the steps in this claim, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately.

89. Accordingly, it is my opinion that claim 2 of the '540 patent is nothing more than a law of nature coupled to well-understood, routine, and conventional activities already engaged in by the scientific community as of 1997, that, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately. It is therefore my opinion that claim 2 is invalid.

3. Claim 8

Claim 8 reads as follows:

> 8. The method according to claim 1, wherein the presence of a foetal nucleic acid from a paternally-inherited non-Y chromosome is detected.

90. This claim specifies that the detected nucleic acid is from a paternally inherited non-Y chromosome.

91. As of 1997, there were many well-understood, routine, and conventional methods for the amplification and detection of fetal nucleic acid from a paternally inherited non-Y chromosome. Indeed, the '540 patent itself references several: Camaschella et al. Blood 1990; Lo et al. Lancet 1993; Bennett et al. N Engl J Med 1993; Bianchi et al. Am J Obstet Gyn 1994; Geifman-Holtzman et al. Am J Obstet Gyn 1996; Aubin et al. Br J Hematol 1997. Furthermore, it was well-understood at that time that a fetus inherits half of its DNA from its mother and half from its father, and that the father's non-Y chromosome nucleic acid, *i.e.*, non-Y chromosome nucleic acid that could be paternally inherited, could be determined through a process known as "genotyping." (*See, e.g.*, Ex. 2 at 3:20-24 ("This application will require the prior genotyping of the father and mother using a panel of polymorphic markers and then an allele for detection will be chosen which is present in the father, but is absent in the mother.").) Moreover, a number of non-Y chromosome nucleotide sequences were available through the GenBank sequence database.

92. For these reasons, the steps recited in claim 8 add nothing that was not well-understood, routine, and conventional as of 1997.

93. Consequently, I conclude that claim 8 of the '540 patent is nothing more than a law of nature coupled to well-understood, routine, and conventional activities already engaged in by

the scientific community as of 1997, that, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately. It is therefore my opinion that claim 8 is invalid.

4. Claims 19 and 20

Claims 19 and 20 of the '540 patent read as follows:

> 19. The method according to claim 1, wherein the sample contains foetal DNA at a fractional concentration of total DNA of at least about 0.14%, without subjecting it to a foetal DNA enrichment step.
>
> 20. The method according to claim 19, wherein the fractional concentration of foetal DNA is at least about 0.39%.

94. It is a law of nature that cell-free fetal DNA is present in the serum or plasma from a pregnant female and that the fractional concentration of fetal DNA to total DNA in maternal plasma or serum may vary within an individual pregnant female over time and among pregnant females (Kazakov et al. Tsitologiia 1995; Lo et al. Am J Hum Genet 1998; Zhong et al. Obstet Gynecol 2000; Smid et al. Ann NY Acad Sci 2001; Bauer et al. Prenatal Diag 2006).

95. As a result of natural human physiological processes, the fractional concentration of fetal DNA to total DNA in serum or plasma of pregnant women far exceeds 0.39%, at least from late in the first trimester onwards (Lun et al. Clin Chem 2008; Lo Clin Chem Lab Med 2012; Lo et al. Am J Hum Genet 1998). This is far in excess of the fractions recited in claims 19 and 20.

96. The parameters set forth in claims 19 and 20 are therefore laws of nature in and of themselves. The human body naturally and necessarily produces far in excess of 0.14% or 0.39% fractional concentration of fetal DNA to total DNA in serum or plasma during pregnancy.

97. Consequently, I conclude that claims 19 and 20 of the '540 patent are nothing more than a law of nature coupled to well-understood, routine, and conventional activities already engaged in by the scientific community as of 1997, that, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately. It is therefore my opinion that claims 19 and 20 are invalid.

5. Claims 21 and 22

Claim 21 reads as follows:

> 21. A method of performing a prenatal diagnosis, which method comprises the steps of:

(i) providing a maternal blood sample;

(ii) separating the sample into a cellular and a non-cellular fraction;

(iii) detecting the presence of a nucleic acid of foetal origin in the non-cellular fraction according to the method of claim1;

(iv) providing a diagnosis based on the presence and/or quantity and/or sequence of the foetal nucleic acid.

Claim 22 reads as follows:

22. The method according to claim 21, wherein the non-cellular fraction as used in step (iii) is a plasma fraction.

98. The specified method of performing a prenatal diagnosis in claim 21 relies entirely on well-understood, routine, and conventional methodologies as of 1997, coupled to the law of nature discussed extensively above–*i.e.*, the existence of cell-free fetal nucleic acid in the serum or plasma from a pregnant female. No novel or inventive concepts, processes, or methodologies are specified in claim 21.

99. Blood samples have been collected by doctors long before 1997. Thus, the step of "providing a maternal blood sample" was well-understood, routine, and conventional by 1997. There is no disclosure in the '540 patent specification of any methods for "providing a maternal blood sample" that were not well-understood, routine, and conventional as of 1997.

100. Claim 21 contains the step of "separating the sample into a cellular and a non-cellular fraction." A "cellular" fraction means a portion of the sample that contains cells. A "non-cellular" fraction means a portion of the sample that does not contain cells. Both plasma and serum are non-cellular fractions of a blood sample because, by definition, neither contains cells. Separating blood from a maternal blood sample into cellular and non-cellular (*i.e.*, plasma or serum) fractions was a well-understood, routine, and conventional activity for researchers in the field by 1997, as I previously detailed in my analysis of claim 1.

101. This claim also requires "detecting the presence of a nucleic acid of foetal origin in the non-cellular fraction according to the method of claim 1." As detailed above in comments on claim 1, this was a well-understood, routine, and conventional activity for researchers in the field by 1997.

102. Claim 21 differs from claim 1 in that it is directed to "[a] method of performing a prenatal diagnosis," and includes the step of "providing a diagnosis based on the presence and/or quantity and/or sequence of the foetal nucleic acid." Prenatal diagnosis as defined in the '540 patent "covers determination of any maternal or foetal condition or characteristic which is related to either the foetal DNA itself or the quantity or quality of the foetal DNA in the maternal serum or plasma." (Ex. 2 at 2:6-10.)

103. The '540 patent specification does not disclose any newly discovered "foetal nucleic acid" whose presence and/or quantity and/or sequence has diagnostic significance. Instead, the specification points to well established diagnostic measures:

> We envisage that foetal DNA analysis in maternal plasma and serum would be most useful in situations where the determination of foetal-derived paternally-inherited polymorphisms/mutations or genes would be helpful in clinical prenatal diagnosis (Lo et al. 1994). Examples include foetal sex determination for the prenatal diagnosis of sex-linked disorders, foetal rhesus D status determination in sensitized rhesus negative pregnant women (Lo et al. 1993), autosomal dominant disorders in which the father carries the mutation and autosomal recessive genetic disorders in which the father and mother carry different mutations (Lo et al.1994), e.g., certain hemoglobinopathies (Camaschella et al. 1990) and cystic fibrosis.

(Ex. 2 at 17:35-47.)

104. The claim is broadly directed to providing a diagnosis based on analysis of fetal nucleic acid, which doctors have been doing for many years. For example, prior publications referenced in the '540 patent had already demonstrated the use of nucleic acid amplification and detection methods for prenatal diagnostic approaches on nucleic acid of fetal origin from maternal blood (Lo et al. Lancet 1990; Camaschella et al. Blood 1990; Bennett et al. N Engl J Med 1993; Lo et al. Lancet 1993; Lo et al. Ann NY Acad Sci 1994; Bianchi et al. Am J Obstet Gyn 1994; Bianchi et al. Ped Res 1996; Bianchi et al. Am J Hum Genet 1997; Aubin et al. Br J Hematol 1997), including prenatal diagnosis approaches based on the presence and/or quantity and/or sequence of nucleic acid of fetal origin obtained from fetal cells in the maternal circulation (reviewed in Steele et al. Clin Obstet Gynecol 1996).

105. Claim 22 specifies that the sample is plasma, as opposed to serum. All of the contemplated techniques for preparing and handling plasma were well-understood, routine, and conventional as of 1997. I discuss this state of the art at length in my analysis of claim1.

106. For the reasons I gave in my analysis of claim 1, the steps in claims 21 and 22, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately.

107. Thus, it is my opinion that claims 21 and 22 of the '540 patent are nothing more than a law of nature coupled to well-understood, routine, and conventional activities already engaged in by the scientific community as of 1997, that, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately. It is therefore my opinion that claims 21 and 22 are invalid.

6. Claim 24

Claim 24 reads as follows:

> 24. A method for detecting a paternally inherited nucleic acid on a maternal blood sample, which method comprises:
>
> removing all or substantially all nucleated and anucleated cell populations from the blood sample,
>
> amplifying a paternally inherited nucleic acid from the remaining fluid and subjecting the amplified nucleic acid to a test for the Paternally inherited fetal nucleic acid.

108. Like claim 1, claim 24 is "[a] method for detecting a paternally inherited nucleic acid," which requires "amplifying a paternally inherited nucleic acid" and "subjecting the amplified nucleic acid to a test for the [p]aternally inherited fetal nucleic acid." While this claim refers to "subjecting the amplified nucleic acid to a test for the [p]aternally inherited fetal nucleic acid," there is nothing in the patent specification that suggests that this is any different than "detecting a paternally inherited nucleic acid" as appears earlier in the claim and in claim 1. As detailed above in comments on claim 1, by 1997, these were well-understood, routine, and conventional activities performed by researchers in the field.

109. This claim contains a step of "removing all or substantially all nucleated and anucleated cell populations from the blood sample." Nucleated cells are cells that contain a nucleus. Most human cells are nucleated. Anucleated cells are cells that do not contain a nucleus.

Some cell types in the human body normally lack a nucleus, such as normal adult red blood cells, whereas others may lack a nucleus as a result faulty cell division. Both plasma and serum are, by definition, free of substantially all nucleated and anucleated cells.

110. As discussed in my analysis of claim 1, as of 1997, there were a number of well-understood, routine, and conventional techniques for "removing all or substantially all nucleated and anucleated cell populations from [a] blood sample," *i.e.*, preparing plasma and serum samples. The patent specification does not reveal that any new techniques that were contemplated. Furthermore, when performing a test to detect nucleic acid in plasma or serum, it was well-understood, routine, and conventional for a researcher to prepare samples that removed all nucleated and anucleated cell populations from the blood sample (Mandel & Metais Proc Meetings Biol Soc 1948; Tan et al. J Clin Invest 1966; Kamm & Smith Clin Chem 1972; Leon et al. Cancer Res 1977; Shapiro et al. Cancer 1983; Emanuel & Pestka Gen Anal Tech Appl 1993; Vasioukhin et al. Br J Haematol 1994; Sorenson et al. Cancer Epi Biomarkers 1994; Fowke et al. J Immunol Methods 1995; Chen et al. Nat Med 1996; Nawroz et al. Nat Med 1996).

111. For the reasons I gave in my analysis of claim 1, the steps in claim 24, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately.

112. Thus, it is my opinion that claim 24 of the '540 patent is nothing more than a law of nature coupled to well-understood, routine, and conventional activities already engaged in by the scientific community as of 1997, that, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately. It is therefore my opinion that claim 24 is invalid.

7. Claim 25

Claim 25 reads as follows:

> 25. A method for performing a prenatal diagnosis on a maternal blood sample, which method comprises
>
> obtaining a non-cellular fraction of the blood sample
>
> amplifying a paternally inherited nucleic acid from the non-cellular fraction
>
> and performing nucleic acid analysis on the amplified nucleic acid to detect paternally inherited fetal nucleic acid.

113. Claim 25 is largely a reiteration of claim 21. As I explained in my analysis of claim 21, doctors had been performing prenatal diagnosis on maternal blood samples for many years prior to 1997.

114. This claim also requires "obtaining a non-cellular fraction of the [maternal] blood sample." As discussed earlier, there were many well-understood, routine, and conventional ways to do this by 1997. The patent specification does not reveal any new ways of obtaining a non-cellular fraction of a maternal blood sample. Furthermore, when performing analysis of nucleic acid in plasma or serum, it was well-understood, routine, and conventional for a researcher to prepare samples that removed the cellular fraction from the blood sample (Vasioukhin et al. Br J Haematol 1994; Sorenson et al. Cancer Epi Biomarkers 1994; Fowke et al. J Immunol Methods 1995; Chen et al. Nat Med 1996; Nawroz et al. Nature Med 1996).

115. This claim also requires "amplifying a paternally inherited nucleic acid" and "performing nucleic acid analysis . . . to detect paternally inherited fetal nucleic acid." While this claim refers to "performing nucleic acid analysis . . . to detect paternally inherited fetal nucleic acid," there is nothing in the patent specification that suggests that this is any different than the "detecting" step in claim 1. For the reasons stated in the analysis of claim 1, these were well-understood, routine, and conventional activities performed by researchers in the field as of 1997.

116. For the reasons I gave in my analysis of claim 1, the steps in claim 25, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately.

117. Thus, it is my opinion that claim 25 of the '540 patent is nothing more than a law of nature coupled to well-understood, routine, and conventional activities already engaged in by the scientific community as of 1997, that, when viewed as a whole, add nothing significant beyond the sum of their parts taken separately. It is therefore my opinion that claim 25 is invalid.

C. The '540 Patent Will Inhibit Future Innovation

118. I understand that Sequenom's claim constructions, as explained in its preliminary injunction papers, include within the scope of the '540 patent any test that amplifies and detects a paternally inherited nucleic acid of fetal origin, regardless of the method for amplification or detection used. I further understand that Sequenom's claim constructions cover tests that amplify

both maternally and paternally inherited nucleic acids, and tests in which the paternally inherited nucleic acid is not differentiated from other fetal nucleic acid or from maternal nucleic acid that is detected.

119. Under Sequenom's claim constructions, and any other claim constructions that would encompass similarly broad scope, the '540 patent would obstruct all research by others into prenatal diagnosis based on cell-free fetal nucleic acid naturally found in maternal serum or plasma. I cannot conceive of any useful procedure involving this subject matter that would not be encompassed by the claims so interpreted.

120. I believe this opinion is consistent with the statements publicly made by Sequenom. I have been informed that Sequenom's Director and Executive Vice President of Research and Development stated in a telephone conference with investors that "we believe [the '540 patent] is the underpinnings of this whole field, and potentially believe anybody who [is] developing, an approach that interrogates the circulating [cell-free] DNA is infringing this key patent in the field." (Sequenom, Inc. 2011 Analyst and Investor Day Conference Call, November 14, 2011, Ex. 10 at p. 4.) While this statement is couched in terms of Sequenom's "potential" belief, it communicates that the patent is considered to be broad enough to block all development efforts. That position is in accord with the broad claim interpretations set forth by Sequenom in its preliminary injunction papers.

121. If the court were to accept Sequenom's arguments, or any claim constructions that would result in a similarly broad interpretation of the claims, the '540 patent would block all clinically useful developments in the field of prenatal diagnosis that are based on cell-free fetal nucleic acid naturally found in maternal serum or plasma.

X. The '540 Patent Does Not Enable the Use of Quantitative Analysis Techniques for Detection of Down Syndrome

122. I understand that Sequenom's preliminary injunction papers explain that claims 1, 2, 8, 19-22, 24, and 25 of the '540 patent are broad enough to cover the use of quantitative nucleic acid analysis techniques to determine whether a fetus has an extra chromosome 21, a condition that causes Down syndrome. It is my opinion that the '540 patent specification would not have

enabled a person of skill in the art in 1997 to use quantitative nucleic acid analysis techniques to determine whether a fetus has an extra chromosome 21, or any other aneuploidy, without undue experimentation. Therefore, it is my opinion that the claims are invalid.

A. Enablement

123. For purposes of my analysis, I have been asked by counsel to assume that the following legal standards apply.

124. I understand that the specification must describe the claimed invention, as well as the manner and process of making and using it in such terms as would enable a person of ordinary skill in the art to make and use the invention, without needing to engage in undue experimentation. I understand that the test of enablement is not whether any experimentation is necessary, but whether any necessary experimentation is undue. If a very difficult and time consuming amount of experimentation is needed to determine how to practice the invention, then this great quantity of experimentation should be considered in the overall analysis. Time and difficulty of experiments are not determinative, however, if they are merely routine.

125. I understand that there is a lack of enablement where information is missing about one or more essential parts or relationships between parts for which one skilled in the art could not develop a solution without undue experimentation. However, because descriptions in patents are addressed to those skilled in the art to which the invention pertains, I understand that a patent applicant need not expressly set forth in the patent specification subject matter which is commonly understood by persons skilled in the art.

126. I understand that to show lack of enablement, it is not necessary to prove an intent by the patent applicant to withhold any particular information. Rather, I understand that all that is required is a failure to teach how to practice the full scope of the invention claimed in the patent.

B. Detection of Down Syndrome Would Have Required Undue Experimentation

127. In order to understand why detection of aneuploidies using cell-free fetal nucleic acid was not feasible in 1997, it is necessary to understand the difference between qualitative and quantitative nucleic acid analysis approaches, and the limitations of the quantitative approaches available in 1997.

128. Qualitative nucleic acid analysis approaches permit the determination of the presence or absence of a particular nucleic acid sequence–is or is not present–in the nucleic acid sample under evaluation. It provides a binary answer of positive or negative. For instance, most people are familiar with the ABO blood group system. There are many other human blood group systems, including the Rh (or Rhesus) blood group system. Under the Rh blood group system, people can be classified as Rh-positive or Rh-negative. Most Rh-negative individuals have a complete deletion of both alleles of a gene called the RhD gene, which encodes the RhD protein. This protein produces an antigen–a molecule that causes the production of antibodies–on the surface of blood cells that can be identified through blood tests. When the mother is RhD-negative due to the deletion of both alleles of the RhD gene, qualitative approaches can be used for the detection of paternally inherited fetal RhD gene sequences in the maternal blood (Lo et al. Br J Haematol 1994). Because the mother does not have any RhD gene sequences, any RhD sequences detected in the maternal blood will be from the fetus, and will confirm that the fetus is Rh-positive. It is important for a woman who is Rh negative to know whether the fetus is Rh positive because the two blood types are incompatible, which can cause problems with the fetus.

129. As highlighted in this example, qualitative techniques are sometimes sufficient where a nucleic acid sequence is known to be absent from the maternal genome. This is because one does not need to know the particular quantity of the nucleic acid. With respect to qualitative techniques, the fact that the sequence is present at all tells you what you need to know.

130. Some disorders, however, cannot be detected so simply. For instance, Down syndrome involves nucleic acid sequences that will be present in a healthy, normal fetus. As I described earlier in this declaration, in almost all cases, Down syndrome is caused by inheriting an extra chromosome 21 from the mother. This extra chromosome is not defective in any way, it is a normal chromosome 21, albeit one more than is needed. Thus, there is no simple way to distinguish it from the other two copies of chromosome 21 that all healthy fetuses have. Instead, a test for Down syndrome must be able to assess the number of occurrences of the relevant sequences in order to identify the anomaly. Such approaches are called quantitative because the accuracy of the count matters.

131. Quantitative nucleic acid analysis approaches permit a determination of the relative abundance of a particular nucleic acid sequence compared to other nucleic acid sequences in a sample under evaluation. For instance, quantitative nucleic acid analysis approaches can achieve a highly accurate determination of the relative abundance of paternal and maternal nucleic acid contributions in a sample containing a mixture of paternally inherited and maternally inherited nucleic acids. Early quantitative nucleic acid analysis approaches were relatively inaccurate. To illustrate, based on quantitative nucleic acid analysis approaches available in 1997, it was originally believed that cell-free fetal DNA was present at a mean concentration of 3%-6% in maternal plasma (Lo et al. Am J Hum Genet 1998). By 2008, subsequent research using newly developed quantitative analysis technology showed that the actual concentration might be around 10%, almost double the amount originally detected (Lun et al. Clin Chem 2008).

132. One approach to quantitative nucleic acid analysis involves determining the order of nucleotides in the nucleic acid. This is referred to as nucleic acid "sequencing." When DNA is sequenced it is generally broken into small pieces first, called "fragments." Nucleic acid sequencing began in the early 1970s, but it was labor intensive and hazardous, and it took considerable effort to sequence even small fragments of nucleic acid.

133. Sequencing techniques have progressed rapidly in recent years. Over the past six to seven years, technology has been developed that allows hundreds of thousands to millions of nucleic acid sequences to be determined simultaneously and quickly. These new sequencing techniques are commonly called "Next Generation." Early nucleic acid sequencing techniques are generally referred to as "First Generation."

134. Among the most obvious differences between Next Generation and First Generation sequencing approaches are the massive amounts of sequence data that can be generated in a short period of time and the dramatically reduced costs of generating that data with Next Generation approaches.

135. Below is the title and abstract of a 2008 commentary by Stephan Schuster that was published in the journal Nature Methods concerning the transformative and enabling aspects of Next Generation sequencing approaches:

Next-generation sequencing transforms today's biology

Stephan C Schuster

A new generation of non-Sanger-based sequencing technologies has delivered on its promise of sequencing DNA at unprecedented speed, thereby enabling impressive scientific achievements and novel biological applications. However, before stepping into the limelight, next-generation sequencing had to overcome the inertia of a field that relied on Sanger-sequencing for 30 years.

(Ex. 11 at p. 16.) As explained in Schuster's article, the technological breakthrough occurred in 2005:

> The first signs of what might revolutionize the sequencing market appeared in 2005 with the landmark publication of the sequencing-by-synthesis technology developed by 454 Life Sciences and the multiplex polony sequencing protocol of George Church's lab.

(*Id.*) The pace of research and development in life sciences was greatly accelerated by Next Generation sequencing.

136. Both Ariosa's Harmony Prenatal Test and Sequenom's MaterniT21 Plus test use Next Generation sequencing techniques to quantify the amount of cell-free nucleic acid from chromosome 21 in the sample being analyzed. As explained above, determining whether a fetus has Down syndrome requires quantitative analysis, not qualitative analysis. Thus, the relevant question is whether the '540 patent specification actually enabled quantitative analysis of aneuploidies such as Down syndrome, wherein it is critical to have an accurate count of the number of sequence occurrences.

137. To answer this question, one does not need to look any further than Dr. Lo's own publications. Dr. Lo recently explained that it was not possible to test for Down syndrome using cell-free fetal nucleic acid in maternal serum or plasma until 2007, a decade after the patent application was filed, and only after Next Generation quantitative sequencing approaches were developed. In a 2012 review article in the journal Clinical Chemistry and Laboratory Medicine Dr. Lo writes:

> The prenatal screening for Down syndrome is perhaps the most sought-after goal for research into non-invasive prenatal diagnosis. However, the development of this application is much more challenging to develop than the detection of fetal sex and RHD genotype. One reason is that chromosome 21, the chromosome that is involved in Down syndrome, is present in both the fetus and the pregnant mother. Furthermore, one has to devise a method for measuring the dosage of the fetal chromosome 21 in the maternal plasma.
>
> * * *
>
> **The first demonstrations that maternal plasma nucleic acids could be used for the direct detection of fetal trisomy 18 and trisomy 21 were achieved in 2006 (11 [Tong et al. 2006]) and 2007 (14 [Lo et al. 2007]), respectively**.

(Ex. 12 at pp. 1-2 (emphasis added).) Dr. Lo explains that a Next Generation sequencing technique called "massively parallel sequencing" finally permitted detection of trisomy 21, the defect associated with Down syndrome:

> With the advent of massively parallel sequencing (18 [Schuster 2008]), one has a method that would allow millions or even billions of DNA molecules to be counted. During massively parallel sequencing, single DNA molecules are spatially separated and then the molecules are clonally amplified and sequenced, or even sequenced directly without amplification. Two groups have shown that massively parallel sequencing would allow fetal trisomy 21 to be detected non-invasively from maternal plasma (19 [Chiu et al. 2008], 20 [Fan et al. 2008]).

(*Id.* at p. 2.)

138. In the same review article Dr. Lo further acknowledges the major limitations of the earlier technologies, such as those described in the '540 patent, when he writes the following:

> With the development of single molecule counting technologies for maternal plasma DNA, new possibilities have also emerged for the non-invasive prenatal diagnosis of fetal monogenic diseases. Thus, one is no longer confined to the detection of DNA sequences that the fetus has inherited from its father but which are absent in its pregnant mother's genome. Instead, one can detect the dosage of a particular mutant gene in the fetus's genome (17 [Chiu et al. 2009], 27 [Lun et al. 2008]).

(Ex. 12 at p. 2.)

139. Dr. Lo provided a similar explanation in a presentation he made to the Royal Society on May 19, 2011, during which he stated that "prior to 2007, most experts working in this

area think that this technology cannot be used for the prenatal diagnosis of Down syndrome and it has actually taken us ten years to solve this problem," and that this was made possible only "over the last two or three years, with the development of Next Generation DNA sequencing . . . ." (Ex. 13 at pp. 1-2.)

140. Accordingly, Dr. Lo's opinion on the matter seems clear–before the advent of Next Generation sequencing technologies, those in the field were not able to use quantitative tests to detect fetal aneuploidies, such as Down syndrome, using cell-free fetal nucleic acid in maternal plasma and serum. He is not alone in his opinion. Numerous publications have acknowledged that the detection of Down syndrome in a fetus using cell-free nucleic acid in maternal plasma and serum was impossible until the development of Next Generation technologies (Fan et al. PNAS 2008; Zimmermann et al. Prenat Diagn 2008; Puszyk et al. Prenat Diagn 2008).

141. My review of the '540 patent and the related literature lead me to the same conclusion. The '540 patent refers to two possible ways of screening for Down syndrome using quantitative nucleic acid analysis techniques. (Ex. 2 at 3:25-51.) The first possible approach described (*id.* at 3:30-43) is based on the notion that the level of cell-free fetal DNA in maternal plasma and serum is higher in pregnancies where the fetus has a chromosomal aneuploidy than in normal pregnancies. Thus, quantitative detection of cell-free fetal nucleic acid in maternal plasma or serum can be used to screen pregnant women for chromosomal aneuploidies. This approach is used in "Example 2" in the patent. (*Id.* at 5:54-8:49.) It is my opinion that a person of skill in the art in 1997 could not have used this approach to detect Down syndrome without undue experimentation. First, Down syndrome is not the only possible aneuploidy that a fetus could possess. Each of the 23 chromosome pairs could potentially fail to separate properly during meiosis resulting in an aneuploidy. Sequenom's own MaterniT21 Plus test, for example, screens for Down syndrome and two other common aneuploidies, trisomy 13 and trisomy 18, which result in Patau syndrome and Edwards syndrome respectively. The '540 patent specification offers no insight into how to distinguish one aneuploidy from another using this approach. Second, the '540 patent also offers no insight into how to rule out other potential causes for increased levels of cell-free fetal nucleic acid in maternal serum or plasma. For example, it is known that the level of cell-

free fetal nucleic acid in the plasma and serum of women suffering from a disorder in pregnancy called preeclampsia is elevated compared to normal pregnancies (Lo et al. Clin Chem 1999). Third, the '540 patent specification notes that this approach "has **the potential** to be used as a new screening marker for foetal chromosomal aneuploidies" and that "[a] large scale study could be carried out to develop cutoff values for screening purposes." (Ex. 2 at 8:35-38 (emphasis added).) This suggests that Drs. Lo and Wainscoat recognized that this approach required a significant amount of experimentation before it could be used to reliably detect fetal aneuploidies, if that was even possible. To date no reliable test for fetal Down syndrome has been developed based on this approach. Finally, as I explained earlier, quantitative nucleic acid analysis approaches were so imprecise in 1997 that the quantity of cell-free fetal DNA measured in maternal plasma varied markedly from the actual quantity of fetal DNA present. Thus, this approach, which relies on accurately detecting the quantity of fetal nucleic acid in a sample, was not feasible until the development of more precise quantitative nucleic acid analysis approaches. Accordingly, it is my opinion that a person with skill in the art in 1997 would not have been able to develop a reliable test for Down syndrome based on this disclosure in the patent specification without undue experimentation.

142. A second possible approach suggested in the '540 patent to screen for Down syndrome was to quantitate the abundance of chromosome 21 from the fetus relative to other fetal chromosomes in maternal serum or plasma. (Ex. 2 at 3:44-51). Cell-free fetal nucleic acid represents only a small fraction of the total cell-free nucleic acid in maternal plasma and serum (Lun et al. Clin Chem 2008). The vast majority of cell-free nucleic acid in maternal plasma and serum belongs to the mother. The amount of cell-free fetal nucleic acid derived from chromosome 21, which is only one of the 23 fetal chromosome pairs, represents an even smaller fraction of the total cell-free nucleic acid in maternal plasma and serum. Thus, when a fetus has an extra chromosome 21 leading to Down syndrome, this extra chromosome will result in only a small change in the total concentration of chromosome 21 in the maternal plasma and serum, which consists of both the mother's chromosome 21 and the fetus's chromosome 21. The ability to detect this incremental increase with precision is necessary to make the second approach described

in the '540 patent possible. This approach was in fact infeasible in 1997 because, as I explained earlier, the nucleic acid analysis techniques available at the time did not have sufficient robustness to determine the number of copies of fetal chromosomes in the mixtures of fetal and maternal cell-free nucleic acid present in the serum or plasma of pregnant females. This approach was impossible until the invention and development of Next Generation sequencing approaches almost ten years later. It is my opinion that almost ten years of experimentation to develop a new technology that would potentially allow a person of skill in the art to use this approach to screen for Down syndrome is undue experimentation.

143. In paragraph 42 of the Evans declaration, Dr. Evans states that "[i]n 1997, various methods for sequencing were known. Since 1997, improved techniques for sequencing have been developed, but the concept of sequencing, that is, identifying the order of the bases, is the same." It is my opinion that this statement is highly misleading. Dr. Evans is correct that nucleic acid sequencing means identifying the order of bases and that definition has not changed since 1997. However, Next Generation sequencing technologies are not merely improved techniques for nucleic acid sequencing. Rather, Next Generation sequencing approaches arose from the development of novel concepts and technologies that were not available or even contemplated as of 1997. Moreover, Next Generation sequencing allows for the analysis of upwards of a million or more single nucleic acid molecules, which is critically required to achieve the precision necessary to detect differences in the relative number of fetal chromosomes when cell-free fetal nucleic acid in maternal plasma or serum is assessed.

144. Accordingly, it is apparent that a person of skill in the art in 1997 would not have been able to use the methods disclosed in the '540 patent specification to detect whether a fetus has an extra chromosome 21, or any other aneuploidy, without undue experimentation.

145. Claims 1, 2, 8, 19-22, 24, and 25 of the '540 patent, the claims I understand Sequenom alleges Ariosa infringes, are all open ended with respect to whether the methods are executed with quantitative or qualitative approaches. I understand that the arguments in Sequenom's preliminary injunction papers indicate that it interprets each of these claims to cover quantitative approaches for the detection of Down syndrome in the fetus. Accordingly, the

foregoing reasoning and conclusion applies to all of these claims under Sequenom's claim constructions, and any other constructions that would encompass quantitative approaches for the detection of Down syndrome or other aneuploidies in the fetus. Therefore, it is my opinion that the claims are invalid.

XI. The '540 Patent is Anticipated by the Kazakov Paper

146. Sequenom's preliminary injunction papers explain that the claims of the '540 patent are broad enough to cover any method which amplifies and detects a paternally inherited nucleic acid, irrespective of whether the method is able to discriminate, enrich, identify, or isolate which nucleic acids are paternally inherited.

147. If this interpretation is accepted, then the procedures described in the paper by Kazakov and his colleagues (Kazakov et al. Tsitologiia 1995) would necessarily fall within the scope of the '540 patent claims as well. As I explain in greater detail below, the Kazakov paper and its methods involved the use of maternal serum and the amplification of cell-free nucleic acid sequences which would have unavoidably amplified paternally inherited fetal sequences. These amplified sequences were then detected and analyzed by gel electrophoresis, a technique I described earlier in this declaration, which involves passing an electric current through a gel containing the nucleic acid sample to separate the nucleic acids by size. While the methods used by Kazakov and his colleagues were not able to distinguish which sequences were paternally inherited, if the '540 patent is so broad that it does not require such, then the methods described in the Kazakov et al. paper would be covered by the claims of the '540 patent.

A. Anticipation

148. For purposes of my analysis, I have been asked to assume by counsel that the following legal standards apply.

149. I understand that a person is not entitled to a patent if the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent. I understand that a person is not entitled to a patent if the invention was patented or described in a printed publication in this or a

foreign country or was in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States.

150. I understand that a patent claim is invalid for a lack of "novelty" (lack of novelty is also called "anticipation") if what is claimed is not new. Anticipation occurs if within the "four corners" of a single prior art reference each and every limitation of the patent claim is disclosed, either explicitly or inherently. I have been informed that a limitation may be inherently disclosed if the missing element would have been necessarily and always present in the prior art device or method, even if the inherently disclosed element was not recognized as present as of the date of the prior art reference.

B. The Asserted Claims as Interpreted by Sequenom are Disclosed in the Work of Kazakov and Colleagues

151. At the time Kazakov and his colleagues were conducting their study, it had already been demonstrated that the amount of cell-free DNA in blood increases as a result of various diseases, such as cancer and lupus (Tan et al. J Clin Invest 1966; Leon et al. Cancer Res 1977; Shapiro et al. Cancer 1983). Kazakov and his colleagues conducted a study on cell-free DNA in the blood of pregnant women to demonstrate that the amount of cell-free DNA in the blood increases during pregnancy. During pregnancy, a fertilized egg grows into a fetus inside the mother's uterus. This process requires the single fertilized egg cell to divide many times to create new cells, a process called cell proliferation, and these cells must also transform into different types of cells such as muscle cells and skin cells, a process called cell differentiation. During this process, many cells die. Kazakov and his colleagues "anticipated that these processes exert an influence on the specifics of the nucleotide composition of the extracellular DNA [*i.e.*, cell-free DNA] in the blood of pregnant women." (Ex. 4 at p. 233.) The results of this study by Kazakov and his colleagues were published in 1995 in an article entitled "Extracellular DNA [*i.e.*, cell-free DNA] in the Blood of Pregnant Women."

152. Kazakov and his colleagues prepared serum samples from men, non-pregnant women, and women in their first and third trimesters of pregnancy, including women with a disorder of pregnancy known as preeclampsia, using standard approaches. (Ex. 4 at p. 233.) Cell-

free DNA was isolated from the serum samples, *i.e.*, all proteins were removed to leave only DNA, by conventional procedures. (*Id.*) DNA is usually isolated before analysis because proteins are known to interfere with DNA analysis. The cell-free DNA samples were then subject to gel electrophoresis, a technique I described earlier in this declaration. (*Id.*) This allowed Kazakov and his colleagues to estimate the concentration of cell-free DNA present in each serum sample. (*Id.*) Next, this cell-free DNA was subjected to DNA amplification using PCR, a technique I described earlier. (*Id.*) The particular sequences that were amplified are known as "Alu repeats." (*Id.*) These are repetitive DNA sequences that occur over a million times in the human genome (Batzer & Deininger Nat Rev Genet 2002, Ex. 14). They are called "Alu repeats" because they can be recognized and cut by an enzyme called Alu I. Over 10% of human DNA consists of Alu repeats. Alu repeats are widely scattered throughout the genome and are present on all human autosomes (*i.e.*, chromosomes 1-22) as well as both the X and Y chromosomes. Three different primers—the short DNA sequences necessary to start each round of the PCR amplification process, and that target specific regions of DNA—called B1, C2, and Tc65 were used to target the Alu repeats. (Ex. 4 at p. 233.) I compared the DNA sequences of these three primers to the Alu repeat sequences, as shown in Exhibit 15, and confirmed that they would have led to the amplification of Alu repeats on every human autosome as well as both the X and Y chromosomes (Batzer & Deininger Nat Rev Genet 2002; Grover et al. Bioinformatics 2002; Ellis et al. Nature 1989; Daniels et al. Nucl Acids Res 1983). The amplified cell-free DNA sequences were then analyzed using gel electrophoresis. (Ex. 4 at p. 233.) This allowed Kazakov and his colleagues to determine the length of the cell-free DNA that had been amplified. (*Id.*) Though serum was used in the Kazakov paper, the authors also suggest that maternal plasma could be used as a source of the cell-free DNA. (*See id.* ("The use of serum instead of plasma for the analysis of the DNA of the blood can be justified if one observes the conditions for formation of a thrombus at room temperature and immediate removal of the serum from the thrombus (Leon et al., 1977; Shapiro et al., 1983). Such was done in the present investigation.").)

153. As a result of this study, Kazakov and his colleagues found that the serum of pregnant women contained more cell-free DNA than the serum of men and non-pregnant women.

(Ex. 4 at pp. 233-34.) They further found that the serum of pregnant women suffering from preeclampsia contained more cell-free DNA than the blood of other pregnant women. (*Id.* at p. 234.) In addition, Kazakov and his colleagues demonstrated that certain Alu repeats, called inter-Alu repeats (which consist of a DNA fragment flanked by two Alu repeats), could only be detected in the blood of pregnant women in their first trimester. (*Id.*)

154. Kazakov and his colleagues believed the increase in cell-free DNA during pregnancy was likely due to nucleic acid excretion by fetal cells. They explain that the fetus undergoes cell proliferation and cell death during pregnancy, which is expected to cause the excretion of fetal DNA:

> According to available data, cellular proliferation, differentiation, and cell death occur in the uterus during pregnancy . . . . It was anticipated that these processes exert an influence on the specifics of the nucleotide composition of the extracellular DNA in the blood of pregnant women.

(Ex. 4 at p. 233.)

155. Kazakov and his colleagues conclude that the increase in cell-free DNA in the blood of women during pregnancy is either fetal or maternal in origin:

> Thus, in the early stages of pregnancy in humans, cells of the fetus (trophoblasts) and the mother (cells of the endometrium and lymphocytes) may excrete DNA.

(Ex. 4 at p. 235.)

156. I interpret the Kazakov paper as teaching that that maternal serum may contain cell-free fetal DNA, since that is one of only two possible explanations it provides.

157. I understand that this analysis is appropriately done on a claim-by-claim basis, which I set forth below. I have also prepared a chart containing my analysis as to each of the asserted claims, which is attached to this declaration as Exhibit 17.

1. Claim 1

Claim 1 reads as follows:

> 1. A method for detecting a paternally inherited nucleic acid of fetal origin performed on a maternal serum or plasma sample from a pregnant female, which method comprises
>
> amplifying a paternally inherited nucleic acid from the serum or plasma sample and

> detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample.

158. As the claims are interpreted by Sequenom, Kazakov et al. (Tsitologiia 1995) described each of these steps. Kazakov and his colleagues reported a method for detecting paternally (and maternally) inherited nucleic acid of fetal origin on a serum or plasma sample from a pregnant female. In the study described in the paper, the authors prepared serum samples from the blood of pregnant females. (Ex. 4 at p. 233.) As explained earlier, Kazakov and his colleagues amplified sequences known as "Alu repeats" (*id.*), which are widely scattered throughout the genome and are present on all human autosomes (*i.e.*, chromosomes 1-22) as well as both the X and Y chromosomes, using PCR. Thus, Alu repeats are present on every chromosome in the fetal genome, both those that are paternally inherited and those that are maternally inherited. They occur on the fetal Y chromosome, if the fetus possesses one, as well as all non-Y fetal chromosomes. By amplifying Alu repeats of cell-free DNA in the serum of pregnant women, which contains cell-free fetal DNA, Kazakov and his colleagues necessarily amplified fetal nucleic acid from a paternally inherited nucleic acid. Kazakov and his colleagues then detected these amplified Alu repeats, including the paternally inherited Alu repeats of fetal origin, using gel electrophoresis. (*Id.*) It is therefore my opinion that claim 1 is invalid.

2. Claim 2

Claim 2 reads as follows:

> 2. The method according to claim 1, wherein the foetal nucleic acid is amplified by the polymerase chain reaction.

159. Claim 2 of the '540 patent is met by Kazakov et al. (Tsitologiia 1995), as the authors did in fact use PCR to amplify the fetal nucleic acid present in maternal serum. (Ex. 4 at p. 233.) It is therefore my opinion that claim 2 is invalid.

3. Claim 8

Claim 8 reads as follows:

> 8. The method according to claim 1, wherein the presence of a foetal nucleic acid from a paternally-inherited non-Y chromosome is detected.

160. Claim 8 is also met by Kazakov et al. (Tsitologiia 1995) as the claims are interpreted by Sequenom. As explained earlier, by amplifying and detecting Alu repeats of cell-free DNA in the serum of pregnant women (Ex. 4 at p. 233), which contains cell-free fetal DNA, Kazakov and his colleagues necessarily detected fetal nucleic acid from a paternally inherited non-Y chromosome. It is therefore my opinion that claim 8 is invalid.

4. Claims 19 and 20

Claims 19 and 20 read as follows:

> 19. The method according to claim 1, wherein the sample contains foetal DNA at a fractional concentration of total DNA of at least about 0.14%, without subjecting it to a foetal DNA enrichment step.
>
> 20. The method according to claim 19, wherein the fractional concentration of foetal DNA is at least about 0.39%.

161. The serum samples containing cell-free fetal DNA used by Kazakov et al. (Tsitologiia 1995) would have had at least the fractional concentrations of fetal DNA to total DNA specified in claims 19 and 20. Kazakov and his colleagues performed their study on serum from pregnant women in the first and third trimesters of pregnancy, including pregnant women suffering from preeclampsia. (Ex. 4 at p. 233.) It is well established that the physiology of normal pregnant human females gives rise to a fractional concentration of cell-free fetal DNA to total DNA in serum or plasma far in excess of 0.39%, at least from late in the first trimester onwards (Lun et al. Clin Chem 2008; Lo Clin Chem Lab Med 2012; Lo et al. Am J Hum Genet 1998). Furthermore, as Kazakov and his colleagues demonstrated, the fractional cell-free fetal DNA concentration to total DNA in the serum of pregnant women suffering from preeclampsia is higher than in normal pregnancies. Thus, the fractional concentration of cell-free fetal DNA to total DNA in the serum or plasma of pregnant women with preeclampsia also much exceeds 0.39%. It is therefore my opinion that claims 19 and 20 are invalid.

5. Claim 21

Claim 21 reads as follows:

> 21. A method of performing a prenatal diagnosis, which method comprises the steps of:
>
> (i) providing a maternal blood sample;

> (ii) separating the sample into a cellular and a non-cellular fraction;
>
> (iii) detecting the presence of a nucleic acid of foetal origin in the non-cellular fraction according to the method of claim1;
>
> (iv) providing a diagnosis based on the presence and/or quantity and/or sequence of the foetal nucleic acid.

162. The Kazakov et al. (Tsitologiia 1995) paper meets each of these steps, as Sequenom interprets this claim. As explained previously, a "cellular" fraction means a portion of the sample that contains cells. A "non-cellular" fraction means a portion of the sample that does not contain cells. Kazakov and his colleagues obtained blood samples from pregnant females and separated each maternal blood sample into cellular and acellular fractions, the acellular fraction in this case being serum. (Ex. 4 at p. 233.) As I described in my analysis of claim 1, Kazakov and his colleagues detected the presence of nucleic acid of fetal origin in the non-cellular fraction using gel electrophoresis. (*Id.*) Kazakov and his colleagues also made a diagnosis of preeclampsia based upon the increase in cell-free DNA, postulating that the increase came from the fetus. (*Id.*)

163. Prenatal diagnosis as defined in the '540 patent "covers determination of any maternal or foetal condition or characteristic which is related to either the foetal DNA itself or the quantity or quality of the foetal DNA in the maternal serum or plasma," including "detection and monitoring of pregnancy-associated conditions such as pre-eclampsia." (Ex. 2 at 2:6-14.) Kazakov and his colleagues were able to provide a diagnosis of preeclampsia based on the increased quantity of cell-free fetal nucleic acid in maternal serum.

164. It is therefore my opinion that claim 21 is invalid.

6. Claim 22

Claim 22 reads as follows:

> 22. The method according to claim 21, wherein the non-cellular fraction as used in step (iii) is a plasma fraction.

165. This claim merely specifies the use of plasma as the non-cellular fraction.

166. Though serum was used by Kazakov and his colleagues, I interpret the Kazakov paper as suggesting that either maternal plasma or serum can be used as a source of the cell-free

DNA. Specifically, the authors explain that "[t]he use of serum instead of plasma for the analysis of the DNA of the blood can be justified if one observes the conditions for formation of a thrombus at room temperature and immediate removal of the serum from the thrombus (Leon et al., 1977; Shapiro et al., 1983). Such was done in the present investigation." (Ex. 4 at p. 233.) Kazakov and his colleagues thus explain their rationale for using serum, and indicate that plasma would be a suitable alternative. It is therefore my opinion that claim 22 is invalid.

7. Claim 24

Claim 24 reads as follows:

> 24. A method for detecting a paternally inherited nucleic acid on a maternal blood sample, which method comprises:
>
> removing all or substantially all nucleated and anucleated cell populations from the blood sample,
>
> amplifying a paternally inherited nucleic acid from the remaining fluid and subjecting the amplified nucleic acid to a test for the Paternally inherited fetal nucleic acid.

167. This method was performed by Kazakov et al. (Tsitologiia 1995), again applying Sequenom's interpretation of the claims. Nucleated cells are cells that contain a nucleus. Most human cells are nucleated. Anucleated cells are cells that do not contain a nucleus. Some cell types normally lack a nucleus, such as normal adult red blood cells, whereas others lack a nucleus as a result faulty cell division. Serum, which is the sample on which Kazakov and his colleagues performed their experiment (Ex. 4 at p. 233), does not contain nucleated or anucleated cells. Kazakov and his colleagues prepared serum samples from maternal blood samples. (*Id.*) As I already discussed in my analysis of claim 1, Kazakov and his colleagues amplified and detected paternally inherited nucleic acid in the serum when this claim is interpreted as broadly as Sequenom proposes. While this claim refers to "subjecting the amplified nucleic acid to a test for the [p]aternally inherited fetal nucleic acid," there is nothing in the patent specification that suggests that this is any different than "detecting a paternally inherited nucleic acid" as appears earlier in the claim and in claim 1. It is therefore my opinion that claim 24 is invalid.

8. Claim 25

Claim 25 reads as follows:

25. A method for performing a prenatal diagnosis on a maternal blood sample, which method comprises

obtaining a non-cellular fraction of the blood sample

amplifying a paternally inherited nucleic acid from the non-cellular fraction

and performing nucleic acid analysis on the amplified nucleic acid to detect paternally inherited fetal nucleic acid.

168. This claim (as interpreted by Sequenom) is also met by the Kazakov et al. (Tsitologiia 1995) paper. A non-cellular fraction of a blood sample is a portion of the sample that does not contain cells. Serum, which was used in the experiment of Kazakov and his colleagues (Ex. 4 at p. 233), is a non-cellular fraction of a blood sample. As I already discussed in my analysis of claim 1, Kazakov and his colleagues amplified and detected paternally inherited nucleic acid in the serum when this claim is interpreted as broadly as Sequenom proposes. While this claim refers to "performing nucleic acid analysis . . . to detect paternally inherited fetal nucleic acid," there is nothing in the patent specification that suggests that this is any different than the "detecting" step in claim 1. It is therefore my opinion that claim 25 is invalid.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

Executed on May 14, 2012 in Ann Arbor, Michigan.

Dr. Eric R. Fearon